IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: **Letts et al**

Application No: **10/718,060** Group Art Unit: **1626**

Filed: **June 10, 2003** Examiner: **Rebecca L. Anderson**

For: **Nitrosated and/or Nitrosylated Cyclooxygenase-2 Inhibitors, Compositions and Methods of Uses**

Attorney Docket No: **102258.157 US1**

Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C. F. R. §1.132

I, David S. Garvey, Ph.D. declare the following:

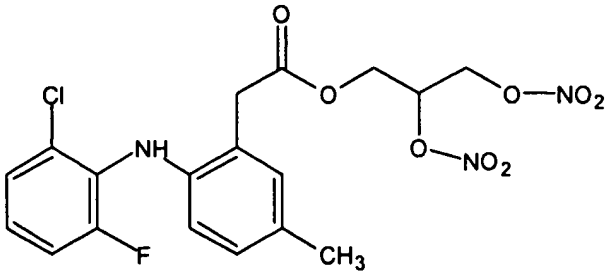
1. I am currently the Executive Project Director and Chief Chemistry Advisor at NitroMed, Inc. (NitroMed). From 1997 to 2003, I was the Senior Director of Chemistry at NitroMed. From 1994 to 1997, I was the Director of Chemistry at NitroMed.

2. I am a co-inventor of U.S. Application No. 10/718,060, filed June 10, 2003 (hereafter "the present application").

3. I have reviewed the specification and claims of the present application, the restriction requirement dated June 14, 2005, the Office Communication dated September 19, 2005 and the PCT patent application WO 99/11605.

4. In the present application, the species elected by the Applicant in the restriction requirement dated July 8, 2005, is the nitrosated cyclooxygenase inhibitor of Formula (V), 2,3-bis(nitroxy)propyl 2(2-((2-chloro-6- fluorophenyl)amino)5-methylphenyl)acetate.

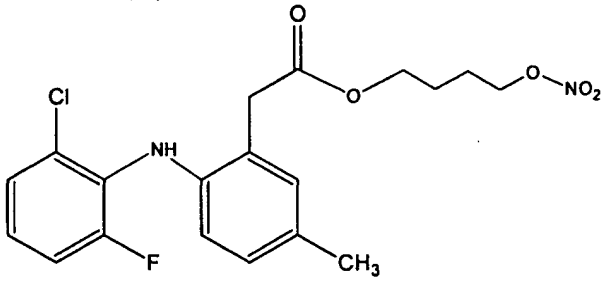
The compound of Formula (V) has the structure:



(V)

5. In the present application, the specification at page 20, lines 11-13, specifically excludes nitrooxy lower alkyl esters, such as 4-(nitrooxy)butyl {2-[(2-chloro-6-fluorophenyl)amino]-5-methylphenyl}acetate of Formula (A), as disclosed in WO 99/11605.

The compound of Formula (A) has the structure:



(A)

6. I understand that the U.S. Patent and Trademark examiner has asserted that the Applicant's election of species is improper because the examiner believes that Applicant specifically excludes the elected species by the proviso on page 20 of the specification. Based on my reading of the specification of WO 99/11605 and on my understanding of the level of ordinary skill in the art of organic chemistry and my knowledge and experience of those skilled in the art, it is my opinion that one of skill in the art would appreciate that the bis-nitrooxy species elected by Applicants is not a nitrooxy lower alkyl ester encompassed by the proviso.

7. It is my opinion that in the present application by excluding nitrooxy lower alkyl esters, Applicants **did not** exclude substituted nitrooxy lower alkyl esters, such as nitrooxy lower alkyl ester compounds substituted with one or more nitrooxy groups, such as, for example, bis(nitrooxy) lower alkyl ester compounds, and the like, or poly(nitroxy) lower alkyl ester compounds, and the like.

8. It is my opinion that one skilled in the art, from reading the specification of WO 99/11605, would appreciate that its disclosure of nitrooxy lower alkyl esters does not encompass substituted nitrooxy lower alkyl esters. In the specification of WO 99/11605, a lower alkyl group is specifically defined as a group that contains up to 7 carbon atoms, preferably 1 to 4 carbon atoms and represents for example methyl, ethyl, propyl or butyl and may be straight chain or branched. (*See* specification at, for example, page 4, lines 17-18). The definition of a lower alkyl group does not contemplate a substituted lower alkyl group. Additionally there are no examples or teaching of a substituted lower alkyl group in the specification of WO 99/11605.

9. It is also my opinion that one skilled in the art would appreciate that a nitrooxy lower alkyl ester compound is a mono(nitroxy) compound that contains only one nitrooxy group and does not encompass a bis(nitroxy) alkyl ester compound that contains two nitrooxy groups or other poly(nitrooxy) alkyl ester compounds that contain more than one nitrooxy group.

10. Furthermore, it is my opinion that one skilled in the art would appreciate that nitrooxy lower alkyl ester compounds do not encompass both nitrooxy lower alkyl ester compounds containing only one nitrooxy group and bis(nitroxy) alkyl ester compounds containing two nitrooxy groups because nitrooxy compounds containing only one nitrooxy group have different biological properties than bis(nitroxy) compounds. For example, recent studies by Young et al, *Biochemical Pharmacology* 70: 1343-1351 (2005), a copy of which is attached hereto, showed that a bis(nitroxy) alkyl ester of naproxen, NMI-1182, produces more absolute NOx, mainly as a nitrite, than a nitrooxy alkyl ester of naproxen containing only one nitrooxy group, AZD3582, that produces NOx mainly as a nitrate. For the Examiner's convenience the experiment conducted by Young et al is described below.

11. The chemical structures of naproxen, NMI-1182 and AZD3582 are presented in **Figure 1** of Young et al:

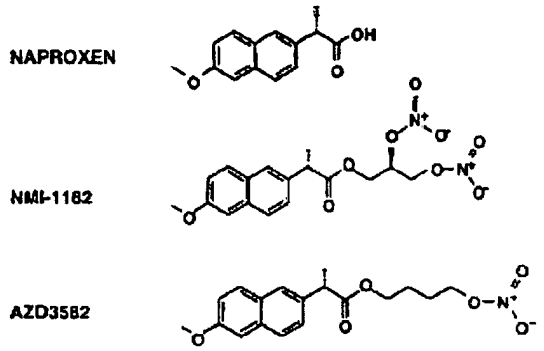


Figure 1

12. NMI-1182 and AZD3582 (70 μM) or DMSO (0.1%, as vehicle control) were incubated in reaction mixtures (total volume of 300 μL) consisting of human liver S9 homogenates (10 mg protein/ml) in 50 mM Tris HCl, pH 7.4 buffer supplemented with reduced glutathione (1 mM) and a NADPH regenerating system (NRS), comprised of 425 μg/ml of ß-NADP, sodium salt, 1.95 mg/ml of ß–D-glucose-6-phosphate, sodium salt, and 375 munits/ml of glucose-6-phosphate dehydrogenase in 0.5 % $NaHCO_3$. The NRS formulation was taken from the In Vitro Technologies (supplier of microsomes and S9) protocol "Instructions for Using Microsomes and S9 Fractions" (Nov. 27, 2001). The reactions in open microfuge tubes were incubated in a 37°C, ambient atmosphere incubator on a gently shaking platform shaker for the indicated times. To terminate the NOx formation reactions, the reaction tubes were capped, placed in boiling water baths for 5-10 minutes, and stored frozen at -80°C. Prior to assay, the tubes were centrifuged at 15,000 x g for 10 min at 4°C and the supernatants filtered through Millipore (Bedford, MA) 10K mw cutoff centrifuge filter units. The filtrates were assayed for nitrite alone and nitrate separately (by reduction of nitrate to nitrite with a bacterial nitrate reductase) to yield total NOx (nitrite/nitrate) via the Greiss Reaction using the Nitrate/Nitrite Colorimetric Assay Kit supplied by Cayman Chemical Co. (Ann Arbor, MI). Results were expressed after subtracting the vehicle control (0.1% DMSO) values, which represented basal nitrate levels.

13. **Figure 11** in Young et al shows the total NOx and nitrite produced from NMI-1182 and AZD3382 after incubation with the human liver S9 homogenate using the protocol

described in paragraph no. 11. The x axis corresponds to the time in hours[1]. The y axis corresponds to the amount of NOx and nitrite produced as millimolar (mM). The results were the averages ± SEM of three experiments (each performed in triplicate); $^{**}p<0.01$ and $^{***}p<0.001$ comparing total NO_x from NMI-1182 vs. AZD3582 (open symbols) or nitrite released by NMI-1182 vs. AZD3582 (closed symbols) at the indicated times, by Two Way ANOVA, Bonferroni Post Test.

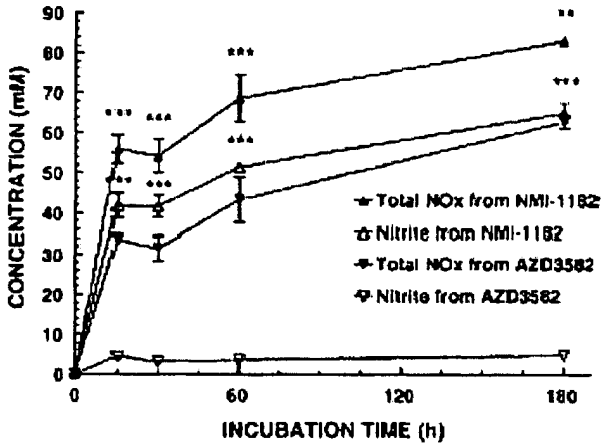


Figure 11

14. As can be seen from the results in **Figure 11**, NMI-1182, a bis(nitrooxy) compound produced more absolute total NOx than AZD3582. Additionally most of the total NOx produced from NMI-1182 was in the form of nitrite while that produced from AZD3582 was in the form of nitrate.

15. It is my opinion that the data from Young et al shows that a nitrooxy alkyl ester compound containing only one nitrooxy group (i.e. AZD3582) has different biological properties than a bis(nitroxy) alkyl ester compound containing two nitrooxy groups (i.e. NMI-1182).

16. In summary, based on the teachings in WO 99/11605 and in Young et al, it is my opinion that one skilled in the art would conclude that the compound of Formula (V) of the present application is **not** encompassed by the nitrooxy lower alkyl esters, such as the 4-

[1] Based on the other experiments reported in Young et al, the incubation time in Figure 11 should be minutes and not hours (h).

(nitrooxy)butyl {2-[(2-chloro-6-fluorophenyl)amino]-5-methylphenyl}acetate of Formula (A) disclosed in WO 99/11605.

17. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity or enforceability of this application or any patent issued thereon.

David S. Garvey, Ph.D. Oct. 17, 2005
Date